

Amendments to the Specification

Please amend the specification as follows:

Please replace the paragraph beginning on page 15, line 15 with the following amended paragraph:

DNA fragments containing the *AccI*-*NcoI* region (Fig. 1) were digested with either *NcoI*, *HinfI* or *Sau96I* (New England Biolabs). These fragments were end-labeled in reactions of 50 μ l containing 50 mM Tris-HCl (pH 7.2), 10 mM $MgCl_2$, 0.1 mM dithiothreitol, 50 μ g/ml BSA, 10 μ Ci $\alpha^{32}P$ dNTP (Amersham--where x represents the correct nucleotide for fill-in), 2 units *E. coli* DNA polymerase large fragment (New England Biolabs). Following labeling, single-stranded material was prepared by electrophoresis. Samples were denatured in 30% dimethyl sulfoxide, 1 mM EDTA and 0.05% bromophenol blue at 90°C for 2 hr. Samples were chilled and electrophoresed in acrylamide gels in a Bethesda Research Labs apparatus. DNA was detected by autoradiography and isolated by elution into 10 mM Tris-HCl (pH 7.0), 1 mM EDTA. Chemical degradation of DNA for sequence analysis was conducted using standard procedures. Cleavage at guanine (G) residues was conducted by reaction with dimethyl sulfonate at 22°C for 10 min. Cleavage at adenine (A) residues was conducted by 12 min reaction at 90°C in 1.5 M NaOH, 1 mM EDTA. Cleavage at cytosine (C) residues was conducted using hydrazine in 2 M NaCl for 13 min at 22°C. Cleavage at thymine (T) residues was conducted using hydrazine with no added NaCl for 10 min at 22°C. Following cleavage, all reactions were twice precipitated using ethanol and thoroughly dried. All samples were reacted with 1 M piperidine at 90°C for 30 min. Piperidine was removed by evaporation in a Savant speed-vac SPEEDVAC concentrator. Fragments were separated by electrophoresis in acrylamide gels (BRL HO apparatus) in 8 M urea, 50 mM Tris-borate (pH 8.3), 1 mM EDTA. Detection of degraded ladder was by autoradiography using Kodak XAR5 film at -70°C.

Please replace the paragraph beginning on page 17, line 2 with the following amended paragraph:

High molecular weight DNA (6 μ g) from tumor MAC117 (see above) was digested with 12 units restriction enzyme *EcoRI* (New England Biolabs) in a volume of 100 μ l for about one hour at 37°C. DNA was obtained by phenol $CHCl_3$ extraction and ethanol precipitation and resuspended in water at a concentration of 0.1 μ g/ml. This DNA (0.2 μ g) was ligated to λ wes λ B

arms (Bethesda Research Labs) (1 µg) using T4 DNA Ligase (New England Biolabs) in a total volume of 20 ml [50 mM Tris-HCl pH 7.4, 10 mM MgCl₂ 10 mM dithiothreitol, 0.5 mM spermidine, 1 mM ATP]. This mixture of ligated DNAs was packaged into infectious bacteriophage particles using the ~~Packagene~~ PACKAGENE system (Promega Biotec). These particles were used to infect bacteria BNN45 and about 8 X 10⁵ individual phage plaques were obtained.

Please replace the paragraph beginning on page 34, line 20 with the following amended paragraph:

This method involves administering to-the patient one of two types of reagent which preferentially binds cells expressing high levels of the protein product of the erbB-related gene described here. These reagents are either antibodies directed against the protein product or a ligand, which is likely to exist because of the homology of the gene to a growth factor receptor. The ligand is isolated by standard techniques using the intrinsic protein kinase activity of the protein product of the erbB-related gene. Extracts of body fluids and cell culture supernatants are incubated with the protein and γ -³²P ATP. Thus, provided is a method of detecting amplification or increased expression of a MAC117 gene relative to normal human mammary tissue by reacting a body sample from a patient diagnosed with cancer with antibodies having specific binding affinity for a least a portion of the MAC117 protein product. The presence of ligand is inferred by incorporation of ³²P into the protein. The ligand is then purified by standard techniques such as ion exchange chromatography, gel permeation chromatography, isoelectric focusing, gel electrophoresis and the like. The natural ligand or antibody is tagged with one or more agents which will cause injury to cells to which they bind. Such tagging systems include incorporation of radioactive or biological toxins. The present discovery of amplification of the erbB-related gene makes it likely that some tumors carry large amounts of the corresponding protein. Hence, the two type-specific agents will bind in larger amounts to the protein present in the body and thus direct the toxic effects of the reagents to these cells.

Please amend the paragraph at page 21, lines 14-23 as follows:

The availability of cloned probes of the MAC117 gene made it possible to investigate its expression in a variety of cell types. The MAC117 probe detected a single 5-kb transcript in A431 cells (Fig. 4). Under the stringent conditions of hybridization utilized, this probe did not detect any

of the three RNA species recognized by EGF receptor complementary DNA. Provided is a method of detecting amplification of a MAC117 gene, wherein the MAC117 gene contains a nucleotide sequence encoding the amino acids encoded by the 423 nucleotides set forth in Figure 1, in mammary tissue from said patient by hybridizing a nucleic acid derived from breast tissue of said patient with a nucleic acid probe of the MAC117 gene, the amplification of said MAC117 gene relative to normal human breast tissue indicating the presence of human mammary carcinoma in said patient. Thus, MAC117 represents a new functional gene within the tyrosine kinase family, closely related to, but distinct from the gene encoding the EGF receptor.

Listing of Claims

Claims 1-57 (Canceled).

58. (Currently amended) A method of diagnosing human mammary carcinoma ~~cancer~~ in a patient comprising:

(a) ~~detecting amplification, rearrangement or increased expression~~ of a MAC117 gene wherein the MAC117 gene contains a nucleotide sequence encoding the amino acids encoded by the following nucleotides: gtctacatgggtgcttccattccaggggatgagctacctggaggatgtgcggc
tcgtacacagggacttgccgctcggaacgtgctggtcaagagtccaacctatgcaaaattacagacttcgggctggctcggctgctgga
cattgacgagacagagtaccatgcagatgggggcaagggttaggtgaaggaccaaggagcagaggaggctgggtggagtgggtgctagc
ccatgggagaactctgagtggccacctccccacaacacacagttggaggacttcctctctgccctcccaggtgcccatcaagtggatggc
ctgaggagtccattctccgccggcggtcaccaccagagtgtgtggtgagttatggtgtgtgtatgggggtgttgggaggggtgggtgag
gagccatgg.

in ~~[[a]]mammary tissue or tumor cell sample~~ from said patient by hybridizing a nucleic acid derived from ~~[[a]]mammary tissue or tumor cell sample~~ of said patient with a nucleic acid probe of the MAC117 gene, the ~~amplification, rearrangement or increased expression~~ of said MAC117 gene relative to normal human mammary tissue indicating the presence of human ~~cancer~~mammary carcinoma in said patient, wherein the nucleic acid derived from the mammary tissue of said patient is DNA or mRNA; ~~or~~

(b) ~~detecting abnormal expression of the protein product of the MAC117 gene by reacting a body sample of said patient with antibodies having specific binding affinity for at least a portion of the protein product, the abnormal expression of said protein product of said MAC117 gene indicating the presence of human cancer in said patient.~~

59. (Currently amended) A method of ~~classifying cancers~~ identifying mammary carcinomas that show amplification of a MAC117 gene comprising:

detecting amplification or increased expression of a MAC117 gene relative to normal human mammary tissue, wherein the MAC117 gene contains ~~either~~ a nucleotide sequence encoding the amino acids encoded by the ~~423~~ following nucleotides:

gtctacatgggtgcttccattccaggggatgagctacctggaggatgtgcggctcgtacacagggacttgccgct
cgggaacgtgctggtcaagagtccaacctatgcaaaattacagacttcgggctggctcggctgctggacattgacga
gacagagtaccatgcagatgggggcaagggttaggtgaaggaccaaggagcagaggaggctgggtggagtgggtgtcta
gcccatgggagaactctgagtggccacctccccacaacacacagttggaggacttcctctctgccctcccaggtgc

ccatcaagtggatggcgctggagtcattctccgccggcggttcacccaccagagtgatgtgtggagttatggtgtg
tgatggggggtgttgggaggggtgggtgaggagccatgg ~~set forth in Figure 1 or the restriction pattern set~~
~~forth in Figure 5A, in [[a]]mammary tissue or tumor sample containing cells from a patient~~
~~diagnosed with cancer, or abnormal expression of a protein product of said MAC117 gene by~~
~~reacting a body sample~~mammary tissue from a patient diagnosed with cancer with antibodies
having specific binding affinity for a least a portion of the MAC117 protein product, and
~~classifying~~identifying those cancers from patients whose ~~body samples show~~mammary
tissue shows amplification ~~or increased expression~~ of said MAC117 gene ~~or abnormal expression~~
~~of the protein product of said MAC117 gene relative to normal human mammary tissue as being~~
~~correlated with amplification of the MAC117 gene or increased expression of the protein product~~
~~of the MAC117 gene.~~

Claim 60 (Canceled).

Claims 61-65 (Not entered).

66. (Cancelled)

67. (Currently Amended) A method of detecting amplification of a MAC117 gene in
mammary tissue, the method comprising:

hybridizing a nucleic acid from the mammary tissue with a nucleic acid probe of the
MAC117 gene, wherein the MAC117 gene contains a nucleotide sequence encoding the amino
acids encoded by the following nucleotides: gtctacatgggtgcttccattccaggggatgagctacctgg
aggatgtgcggctcgtacacagggacttggccgctcggaacgtgctggtcaagagtccaacatgtcaaaattacagacttcgggctggc
tccgctgctggacattgacgagacagagtaccatgcagatgggggcaaggttaggtgaaggaccaaggagcagaggagctgggtgga
gtggtgtctagcccattgggagaactctgagtggccacctccccacaacacacagttggaggacttcctcttctgccctcccaggtgcccatc
aagtggatggcgctggagtcattctccgccggcggttcacccaccagagtgatgtgtggagttatggtgtgtgatggggggtgttgggag
gggtgggtgaggagccatgg.

and wherein the nucleic acid from mammary tissue is DNA or mRNA, and

comparing the copy number of the MAC117 gene in the mammary tissue to the copy
number of normal human mammary tissue, an increase in copy number relative to normal human
mammary tissue indicating amplification of the MAC117 gene.

68. (New) A method of diagnosing human mammary carcinoma in a patient comprising:

(a) detecting increased expression of a MAC117 gene, wherein the MAC117 gene contains a nucleotide sequence encoding the amino acids encoded by the following nucleotides: gtctacatgggtgcttccattccaggggatgagctacctggaggatgtgcggctctacacagggacttgccgc tcggaacgtgctggtcaagagtccaacatgtcaaaattacagacttcgggctggctcggctgctggacattgacgagacagagtaccat gcagatgggggcaaggtaggtgaaggaccaaggagcagaggaggctgggtggagtgggtgtctagcccatgggagaactctgagtggc cacctccccacaacacacagttggaggacttctcttctgccctcccaggtgcccataagtgatggcgtggagtccattctccgccggc gggtcaccaccagagtgatgtgtggagttaggtgtgtgatgggggggtgtgggaggggtgggtgaggagccatgg, in mammary tissue from said patient by hybridizing an RNA derived from mammary tissue of said patient with a nucleic acid probe of the MAC117 gene, the increased expression of said MAC117 gene relative to normal human mammary tissue indicating the presence of human mammary cancer in said patient; or

(b) detecting increased expression of the protein product of the MAC117 gene, wherein the MAC117 gene contains a nucleotide sequence encoding the amino acids encoded by the following nucleotides: gtctacatgggtgcttccattccaggggatgagctacctggaggatgtgcggctctacacagg gacttgccgctcggaaacgtgctggtcaagagtccaacatgtcaaaattacagacttcgggctggctcggctgctggacattgacgaga cagagtaccatgcagatgggggcaaggtaggtgaaggaccaaggagcagaggaggctgggtggagtgggtgtctagcccatgggaga actctgagtggccacctccccacaacacacagttggaggacttctcttctgccctcccaggtgcccataagtgatggcgtggagtcca ttctccgccggcggttcaccaccagagtgatgtgtggagttaggtgtgtgatgggggggtgtgggaggggtgggtgaggagccatgg, by reacting mammary tissue of said patient with antibodies having specific binding affinity for at least a portion of the protein product, the increased expression of said protein product of said MAC117 gene indicating the presence of human mammary carcinoma in said patient.

69. (New) A method of identifying mammary cancers that show increased expression of a MAC117 gene comprising:

detecting increased expression of a MAC117 gene relative to normal human mammary tissue, wherein the MAC117 gene contains a nucleotide sequence encoding the amino acids encoded by the following nucleotides: gtctacatgggtgcttccattccaggggatgagctacctggaggatgtgcgg ctcgtacacagggacttgccgctcggaaacgtgctggtcaagagtccaacatgtcaaaattacagacttcgggctggctcggctgctgg acattgacgagacagagtaccatgcagatgggggcaaggtaggtgaaggaccaaggagcagaggaggctgggtggagtgggtgtctag cccatgggagaactctgagtggccacctccccacaacacacagttggaggacttctcttctgccctcccaggtgcccataagtgatgg

cgctggagtcattctccgccggcggttcacccaccagagtgatgtgtggagttatggtgtgtgatggggggtgtgggaggggtgggtgaggagccatgg,

in mammary tissue from a patient diagnosed with cancer by reacting mammary tissue from a patient diagnosed with cancer with antibodies having specific binding affinity for a least a portion of the MAC117 protein product, and

identifying those cancers from patients whose mammary tissue shows increased expression of said MAC117 gene relative to normal human mammary tissue.

70. (New) A method of detecting overexpression of a MAC117 gene in human mammary carcinoma, the method comprising:

(a) hybridizing MAC117 DNA or mRNA from the tissue with a nucleic acid probe of the MAC117 gene, wherein the MAC117 gene contains a nucleotide sequence encoding the amino acids encoded by the following nucleotides: gtctacatgggtgcttccattccaggggatgagctacctgaggatgtgcggctcgtacacagggacttgccgctcggaacgtgctggtcaagagtccaacatgtcaaaattacagacttcgggctggctcggctgctggacattgacgagacagagtacatgcagatgggggcaaggtaggtgaaggaccaaggagcagaggaggctgggtgga gtggtgtctagcccatgggagaactctgagtggccacctccccacaacacacagttggaggacttcctcttctgcctcccaggtgcccataagtggatggcgctggagtcattctccgccggcggttcacccaccagagtgatgtgtggagttatggtgtgtgatggggggtgtgggagggtgggtgaggagccatgg,

an increase in MAC117 DNA or mRNA relative to normal mammary tissue indicating overexpression of the MAC117 gene, or

(b) contacting the tissue with antibodies having specific binding affinity for at least a portion of the protein product of a MAC117 gene, an increase in antibody binding relative to normal mammary tissue, indicating overexpression of the MAC117 gene.